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PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	SVEN BERGSTROM ET AL.
Serial No.	:	08/375,993
Filed	:	January 20, 1995
For	:	BORRELIA ANTIGEN
Examiner	:	Sidberry
Group Art Unit	:	1802

530 Fifth Avenue
New York, New York 10036

DECLARATION OF DR. JUDY JARECKI-BLACK

Asst. Commissioner For Patents
Washington, D.C. 20231

Sir:

Dr. Judy Jarecki-Black hereby declares and says that:

1. I am advised and therefore believe that the Examiner questions whether a vaccine composition containing OspA or a method of administering OspA as described in this application is enabled, i.e., that such a composition and method can be done as described in the application. I have read and understood a publicly available version of it, namely U.S. Patent No. 5,523,098. Studies of an OspA vaccine were performed under my direction, supervision and control in the ordinary course of business. In particular, attached is a report showing the results of challenge against Lyme disease caused by *Borrelia burgdorferi* five months after administration of an OspA vaccine

composition comprising an immunologically-effective amount of a recombinant *Borrelia* OspA which, I am advised and therefore believe, is in accordance with claims of this patent application, and, from my review, is in accordance with the teachings of this application. The vaccine was protective.

2. I have over 18 years experience in infectious Diseases research at the Medical University of South Carolina (MUSC), the University of Georgia (recipient of NIH fellowship in Molecular Parasitology), at the USDA, and at Rhone Merieux, Inc., mainly human; and in the couse of that research I have worked on Lyme disease for five years. In this experience, my expertise has included developing animal models reasonably predictive of results in humans. I attended medical school and graduate school at The Medical University of South Carolina (Charleston South Carolina) and was awarded a Ph.D. in Molecular and Cellular Biology and Pathobiology in 1988. In view of my education, training and experience, it is my expert opinion that the dog model of Lyme disease is reasonably predictive of results in humans; and, that the protection observed in dogs is reasonably predictive of protection in humans. That is, it is my expert opinion that the fact that the vaccine was protective in dogs is reasonably predictive that it is protective in humans; and, since I am advised and therefore believe that the vaccine and administration thereof was in accordance with claims of this patent application, it is my expert opinion that the claimed

invention is protective in dogs and humans, and enabled by this application.

3. I also declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: 24 Oct 1996

Judy Jarecki-Black, Ph.D.

RESEARCH REPORT: LYME 94/058
DATE: 01 NOVEMBER 1994

TITLE: CANINE LYME DISEASE: DURATION OF IMMUNITY ELICITED WITH A CANINE OSP A MONOVALENT LYME VACCINE

PURPOSE: To determine the duration of immunity (DOI) provided by a monovalent OspA vaccine against canine Lyme Disease.

PROCEDURE:

Thirty-three (33) Beagles were divided into two groups. The first group (20 dogs) received two SC doses of a monovalent vaccine (10 µg/dose OspA) at a 3 or 4 week vaccination interval. The second group was untreated (13 dogs). All dogs were tick-challenged 5 to 6 months after the second vaccination. Antibody levels were determined at regular intervals by ELISA. Vaccine efficacy was assessed by spirochete reisolation at one and two months postchallenge. Dogs were also monitored for clinical signs indicative of canine Lyme Disease (LD).

RESULTS:

1.0 Safety: No adverse local or generalized reactions were found following injection of vaccine.
2.0 Efficacy:

Group	# Dogs	Pre-Challenge Reisolation	Post-Challenge Reisolation	Kidney Biopsy and Joint Smear/Culture Reisolation
Ly	20	19/20 = 95%	1/20 = 5%	2/20 = 10%
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

The monovalent Ly OspA Vaccine:

- elicits protection against canine LD as assessed by both spirochete reisolation and clinical signs.
- this protective response is effective at least five months post vaccination.
- protects against spirochete infection (90%) and clinical signs after a natural challenge.

DATA LOCATION: Notebooks 75 and 88

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RESEARCH REPORT: LYME 94/058
DATE: 01 NOVEMBER 1994

TITLE: CANINE LYME DISEASE: DURATION OF IMMUNITY ELICITED WITH A CANINE OSP A MONOVALENT LYME VACCINE

1.0 PURPOSE:

1.1 To determine if immunity elicited with a monovalent OspA vaccine (designated Ly) can protect dogs against tick-challenge five months after immunization.

1.2 To obtain information concerning the safety of the monovalent Lyme vaccine.

2.0 INTRODUCTION:

Previous experiments have shown that an OspA monovalent vaccine (Ly), administered subcutaneously, provides protection against experimental *Borrelia burgdorferi* infection in dogs (RR LYME 94/041) in a short term efficacy trial. The purpose of the present study was to determine if vaccine-induced immunity is sufficient to protect dogs against a tick-challenge five months after immunization.

3.0 MATERIALS AND METHODS:

3.1 Animals:

Thirty-three Beagle puppies (either sex; nine to ten weeks of age; negative for Lyme and *Leptospira* vaccination) were obtained from Ridglan Farms (Mount Horeb, WI). The puppies were divided randomly into two groups and vaccinated.

1	10	SC	Ly; 4 week interval
2	13		Untreated controls

3.2 Vaccine Preparation:

The OspA monovalent vaccine (designated Ly) was prepared by diluting a stock concentration of the OspA purified protein produced for RMI by Connaught Laboratories. The concentrate, lot #D00814, was manufactured on 04 December 1992 for use in human clinical trials. The concentration of the stock was 465 µg OspA/ml. The Lyme vaccine was produced by diluting the stock concentration in sterile diluent (obtained as a production lot, #02A50) to a concentration of 10 µg/ml of OspA and aliquoting the vaccine into single and multiple use vials (1 ml and 10 ml respectively; lot # 0908931). The vaccine was tested satisfactorily for sterility on 25 October 93).

3.0 MATERIALS AND METHODS: (Continued ..)

3.3 Vaccination Protocol:

Dogs received two doses of vaccine (1 ml/dose) administered subcutaneously at an interval of three (Group 1) or four weeks (Group 2). Signs of anaphylaxis, including difficulty in breathing, itching, and edema, were monitored for the initial 15 minutes following injection. Additionally the animal technicians observed the dogs continuously for the first hour after vaccination, and then at regular intervals during the 14 days after each injection. Signs monitored included swelling, pain, tenderness, and scratching at the injection site. Prior to administration of the second injection, the site of the primary vaccination was palpated for swelling and tenderness.

3.4 Serology:

Blood was taken for titer determination before each vaccination and at monthly intervals thereafter. OspA titers were determined by ELISA, according to the standard procedure used in our laboratory (# 15-012).

3.5 Challenge:

All dogs were challenged with *B. burgdorferi* using naturally infected ticks, according to the challenge procedure of Appel *et al* (1994). The interval between final vaccination and challenge was 24 weeks for Group 1 and 21 weeks for Group 2. The ticks were gathered in Westchester county, NY, an area endemic for Lyme Disease, and the challenge was conducted according to the procedure of Appel *et al* (1994). The *Borrelia burgdorferi* infection rate of these ticks, determined by Dr. Thomas Mather of the University of Rhode Island, was 60%.

3.6 Skin Biopsy and Spirochete Reisolation:

All dogs were biopsied at one and two months postchallenge. The skin around the site of tick attachment was shaved, prepped with betadine surgical scrub, anesthetized with 2% lidocaine injected intradermally, and punch-biopsied using a Baker Skin Punch. Skin samples were placed in tubes containing culture medium (BSK media with heat-inactivated rabbit serum and antibiotics) and transported to the laboratory. Tubes were supplemented with additional media and placed in a candle jar. The jar was incubated for six weeks. Tubes were examined weekly for the presence of spirochetes, using a dark field microscope. At least ten fields were examined using a 40X objective before the sample was considered negative.

MATERIALS AND METHODS: (Continued ..)

Clinical Signs and Symptoms:

Clinical signs of canine Lyme disease (LD) were not expected following infection, due to the variable nature of the disease, therefore efficacy of the vaccines was assessed primarily by the reisolation of *Borrelia burgdorferi* spirochetes from skin biopsy samples. However, all dogs were monitored for appearance of signs indicative of LD. Attachment 1 shows a typical observation sheet filled out daily by animal caretakers. Pain and tenderness, temperature, lameness, ataxia, depression, and anorexia are among the signs for which these dogs were monitored. The attending veterinarian and the Principal Investigator (PI) also observed these dogs at regular intervals, and both were notified by animal caretakers when any adverse event concerning these dogs was noted. Observation of these dogs will continue until 23 November 1994.

4.0 RESULTS:

4.1 Vaccine Safety:

All vaccinated dogs were monitored for adverse reactions (including anaphylaxis) for the first fifteen minutes following vaccination by the PI, and two weeks following each vaccination by the animal caretakers. No adverse reactions were found at any time following injection of either the Lyme monovalent vaccine. Additionally no swelling, pain, tenderness, or itching was found at the injection site during the two week period following vaccination.

4.2 Antibody Titers (See Table 1):

4.2.1 Antibody to OspA was determined by ELISA. Blood was drawn on the 22 November 93 (prebleed), and at monthly intervals thereafter.

4.2.2 Table 1 lists the ELISA values. At the time of tick challenge, most of the dogs vaccinated with the monovalent vaccine (10 µg OspA) still exhibited significant OspA antibody titers. One dog, FAS, failed to mount a significant antibody response to OspA.

4.0 **RESULTS:** (Continued ..)

4.3 Spirochete Reisolation (See Table 1):

4.3.1 Skin biopsies were performed for all dogs at one and two months postchallenge. Biopsies were cultured for six weeks and examined for spirochete reisolation. Spirochetes were reisolated from seven of twelve control dogs at the first biopsy (58%). One sample could not be read because it was lost after five weeks in culture, due to contamination. All 13 samples (100%) from control dogs were positive for spirochetes by the second biopsy date.

4.3.2 Results show that only two dogs vaccinated with the monovalent Lyme vaccine (HVT and DXT) were positive for spirochetes: a reisolation rate of 10%.

4.4 Clinical Signs of Canine Lyme Disease (See Tables 1 and 2):

Five months postchallenge, five of the 13 unvaccinated controls (39%) have experienced episodes attributable to LD; two dogs (HXT and JCT) have had multiple episodes. One of the twenty vaccinates (5.0%) also experienced an episode of lameness (see discussion).

5.0 DISCUSSION:

5.1 Lyme Disease (LD), caused by the pathogenic spirochete *Borrelia burgdorferi*, is currently the most commonly reported tick-borne disease in humans in the United States. Additionally reports of canine are increasing due to the heightened awareness of this syndrome among veterinarians.

5.2 Research has shown that one of the major outer surface proteins of *Borrelia burgdorferi*, designated OspA, is a potent immunogen and provides protection against spirochete infection in a variety of animals (Edelman, 1990; Fikrig et al, 1992). The purified OspA protein, produced in ample amounts by recombinant technology, is the basis of two human vaccines currently undergoing clinical trials (MMWR, 1994). A canine Ly vaccine, with 10 ug/ml of OspA/ml, has also been developed.

5.0 **DISCUSSION:** (Continued ..)

5.3 Vaccine efficacy was assessed by determining the ability of the vaccine to prevent both spirochete dissemination and clinical signs in short term and duration of immunity trials. LD in dogs often does not result in the appearance of clinical disease (Levy and Magnarelli, 1992), therefore in addition to the reporting of clinical signs, vaccine efficacy was based upon the ability of the experimental preparations to prevent spirochete proliferation, as assessed by the reisolation of spirochetes from skin biopsies. Spirochete reisolation is the most important, and most consistent, parameter to consider when assessing the efficacy of a vaccine. If vaccination decreases or eliminates this dissemination, the animal will not develop clinical signs. The Ly vaccine was shown to protect >80% of recipients against spirochete proliferation in the short term efficacy trial (See Research Report Lyme 94/042; submitted to the USDA 19 AUG 94). In the duration of immunity (DOI) study, with dogs challenged 5 to 6 months after vaccination, 100% of the untreated controls were positive for spirochetes by the second biopsy date. In contrast spirochetes were reisolated from only two of the vaccinates (10%).

5.4 Dogs were also observed for clinical signs resulting from infection. These observations were reported by animal technicians (see Materials and Methods), blinded as to the vaccination status of each dog. In the short term efficacy study 25% of the untreated controls demonstrated signs typical of canine Lyme disease (LD), mainly lameness, while no vaccinate was observed with signs. In the DOI study six of the dogs have shown such signs; five are unvaccinated controls (39%) and one dog was a vaccinate (5%). The first episode of lameness was noted approximately five months post-challenge; since then two of the untreated dogs (JRT and IAT) have exhibited recurring episodes.

5.5 One vaccinate, HPS, was reported with swelling and slight lameness in the front right foot approximately 2 months after challenge. The animal was never positive for spirochete isolation, although cultures from that dog were examined specifically with the lameness episode in mind. It is possible that this episode was not the result of canine LD, but was attributable to other causes (trauma, etc.). However, the dog was listed as positive for clinical signs in order to provide as stringent a test as possible for this experimental vaccine.

5.6 Antibody titer results show that all but one of the dogs (FAS) vaccinated with the monovalent vaccine had seroconverted after vaccination, as determined by a difference in prebleed and prechallenge titers of at least two dilutions. This represents a seroconversion rate of 95%. By time of challenge all but two vaccinates (FAS and HVT) still exhibited significant titers. None of the control dogs showed a sustained increase in OspA antibody levels, although three controls (HXT, JBT, and JIT) did have low levels of antibody reported from the prechallenge bleed.

5.0 DISCUSSION: (Continued ..)

The safety of the Ly monovalent vaccine is also demonstrated by the results of this experiment. No adverse effects were noted at the time of vaccination, or in the two week period following each injection. The 10 µg OspA/dose was well tolerated by all of the puppies. Because this vaccine contains no adjuvant, even the mild and transient granulomatous response characteristic of vaccination with most adjuvanted preparations was absent.

6.0 CONCLUSION:

The monovalent vaccine, containing 10 µg OspA/dose:

- Is perfectly safe in 9 to 10 weeks old puppies.
- Is very antigenic and induces a seroconversion in 95% of recipients.
- elicits an immune response which protects vaccinates against spirochete infection (90%) and clinical signs five to six months after vaccination, when 100% of the controls demonstrate spirochete infection and 39% exhibit clinical signs following tick challenge.

7.0 ACKNOWLEDGEMENTS:

We gratefully acknowledge the technical expertise of Massai Jarecki, Mark King, Gordon Gilreath, John Bauman, and Dr. Frank Hypolite.

8.0 REFERENCES:

8.1 Appel, M.J.G. Experimental Lyme Disease in dogs produces arthritis and persistent infection. JID 167: 651-664, 1993.

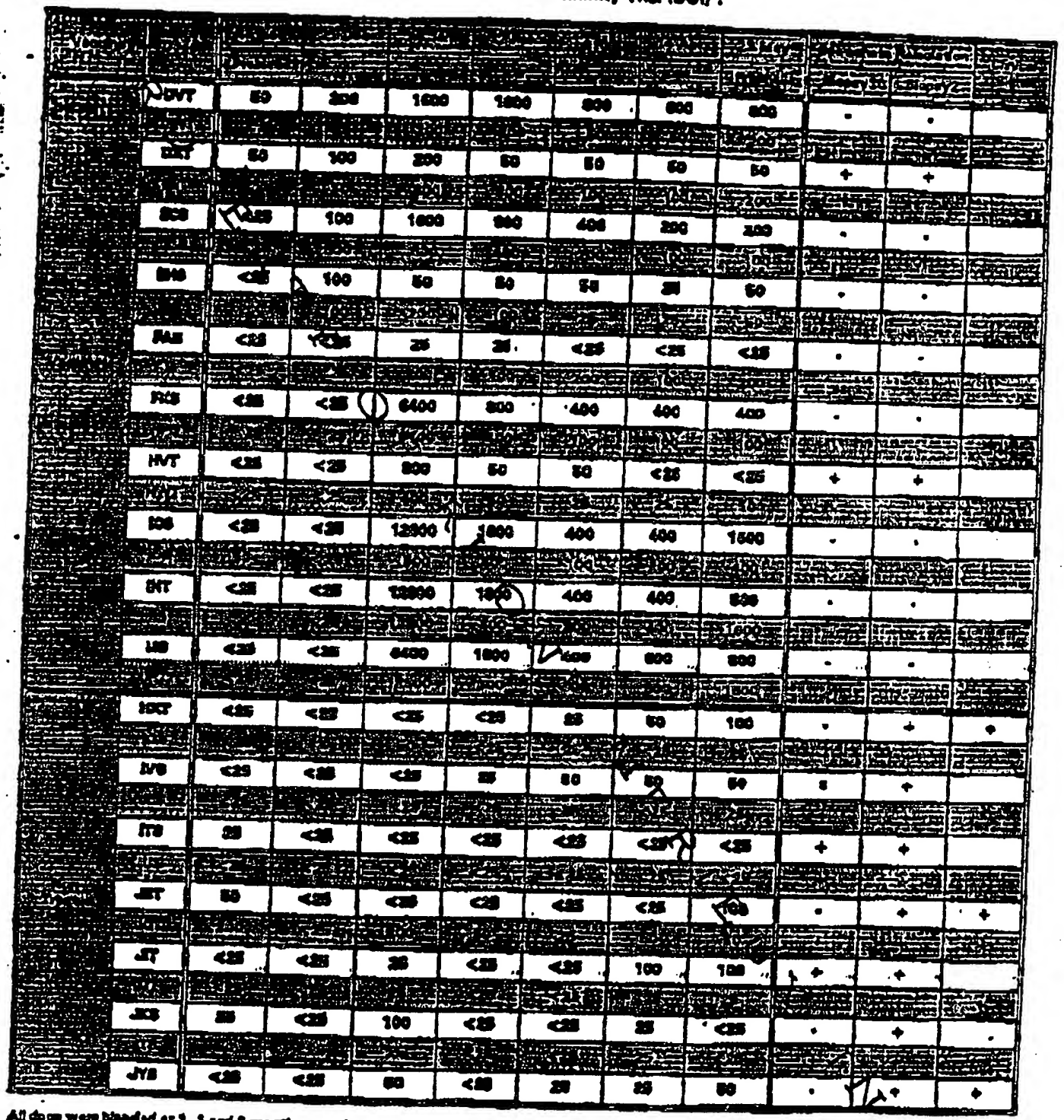
8.2 Edelman, R. Perspective on the development of vaccines against Lyme disease. Vaccine 9: 531-532, 1991.

8.3 Fikrig, E., *et al.* Roles of Osp A, Osp B, and flagellin in protective immunity to Lyme borreliosis in laboratory mice. Inf. Immun. 60: 657-661, 1992.

8.4 Levy, S.A. and L.A. . Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and subsequent development of limb/joint borreliosis. JAVMA 200: 344-347, 1992.

8.5 Lyme Disease - United States, 1993. MMWR 43: 564-572. August 12, 1994.

Table 1. Results of Antibody Titers to Osp A, Spirochete Reisolation and Clinical Signs in the Duration of Immunity Trial (DOI).



All dogs were biopsied at 1, 2 and 3 months postchallenge (see Materials and Methods). Skin samples were cultured in BSK media (supplemented with antibiotics and 10% rabbit serum) for 8 weeks; tubes were examined weekly using a dark field microscope, with at least ten fields examined before a sample was considered as negative.

aSample lost due to contamination

Table 2. Clinical Signs of Canine Lyme Disease in Five-Month Challenge

[illegible]	Control	10-15 Aug	Lameness (all limbs), [illegible], depressed, anorexic.
		15 Sep	Lame (Left Front). All episodes resolved spontaneously
JST	Control	31 Aug	Lame (Rear Legs) resolved spontaneously
JGT	Control		
JJY	Control	24-25 Sep	Lame (Right Front) resolved spontaneously
JYS	Control		
HPG	VAX	15-16 Aug	Limping (Right Front); resolved spontaneously

Dogs were vaccinated with 1 ml Ly vaccine [illegible] at a three or four week interval. Challenge was at 5 to 6 months ([illegible] June 1994). Dogs were monitored blindly for clinical signs by animal technicians.

[illegible]